

Application No.: 10/735019

Docket No.: CL2029USNA

Page 2

Listing of the Claims**RECEIVED
CENTRAL FAX CENTER****AUG 14 2006****Claim 1 (Original).** A bacterial production host comprising:

- a) a plasmid comprising:
 - (i) a target gene to be expressed; and
 - (ii) a replicon controlled by antisense-RNA regulation; and
- b) a mutation in a gene selected from the group consisting of *thrS*, *rpsA*, *rpoC*, *yjeR*, and *rhoL* wherein the nucleotide sequence of the mutated *thrS* gene is SEQ ID NO: 19; the nucleotide sequence of the mutated *rpsA* gene is SEQ ID NO: 21; the nucleotide sequence of the mutated *rpoC* gene is SEQ ID NO: 22; the nucleotide sequence of the mutated *yjeR* gene is SEQ ID NO: 23; and the sequence of the mutated *rhoL* gene is SEQ ID NO: 25.

Claim 2 (Original). A bacterial production host according to Claim 1 wherein the host is *E. coli*.**Claim 3 (Original).** A bacterial production host according to Claim 2 comprising:

- a) a plasmid comprising:
 - (i) a target gene to be expressed; and
 - (ii) a replicon controlled by anti-sense RNA regulation; and
- b) a mutation in a gene selected from the group consisting of *thrS*, *rpsA*, *rpoC*, *yjeR*, and *rhoL* where the mutation of the *thrS* gene is at the 1798679 base of the *E. coli* chromosome; the mutation of the *rpsA* gene is at 962815 base of the *E. coli* chromosome; the mutation of the *rpoC* gene is at 4187062 base of the *E. coli* chromosome; the mutation of the *yjeR* gene is at 4389704 base of the *E. coli* chromosome; and the mutation of the *rhoL* gene is at 3963892 base of the *E. coli* chromosome.

Claim 4 (Original). A bacterial production host according to any of Claims 1-3 wherein the plasmid of step (a) is comprises a replicon selected from the group consisting of p15A and pMB1.**Claim 5 (Original).** A bacterial production host according to any of Claims 1-3 wherein the target gene encodes a polypeptide useful in the production of a genetic end product selected from the group consisting of isoprenoids, carotenoids, terpenoids, tetrapyrroles, polyketides, vitamins, amino acids, fatty acids, proteins, nucleic acids, carbohydrates, antimicrobial agents, anticancer agents, poly-hydroxyalkanoic acid synthases, nitrilases, nitrile hydratases, amidases, enzymes used in the production of synthetic silk proteins, pyruvate decarboxylases, alcohol dehydrogenases, and biological metabolites.

Application No.: 10/735019

Docket No.: CL2029USNA

Page 3

Claim 6 (Original). A bacterial production host according to any of Claims 1-3 wherein the target gene is selected from the group consisting of *crtE*, *crtB*, *crtI*, *crtY*, *crtX* and *crtZ*.

Claim 7 (Original). A bacterial production host according to any of Claims 1-3 selected from the group consisting of *Pseudomonas*, *Shewanella*, *Erwinia*, *Proteus*, *Enterobacter*, *Actinobacillus*, *Yersinia*, and *Pantoea*.

Claim 8 (Original). A bacterial production host according to any of Claims 1-3 wherein the host is an enteric bacteria.

Claim 9 (Original). A bacterial production host according to claim 8 selected from the group consisting of *Escherichia* and *Salmonella*.

Claim 10 (Original). A method for the expression of a target gene comprising:

- a) providing an bacterial production host according to any one of Claims 1-3 comprising a target gene to be expressed;
- b) growing the production microorganism of step (a) under suitable conditions wherein the target gene is expressed.

Claim 11 (Original). A method according to Claim 10 wherein the target gene encodes a polypeptide useful in the production of a genetic end product selected from the group consisting of isoprenoids, carotenoids, terpenoids, tetrapyrroles, polyketides, vitamins, amino acids, fatty acids, proteins, nucleic acids, carbohydrates, antimicrobial agents, anticancer agents, poly-hydroxyalkanoic acid synthases, nitrilases, nitrile hydratases, amidases, enzymes used in the production of synthetic silk proteins, pyruvate decarboxylases, alcohol dehydrogenases, and biological metabolites.

Claim 12 (Original). A method according to Claim 11 wherein the target gene is selected from the group consisting of *crtE*, *crtB*, *crtI*, *crtY*, *crtX* and *crtZ*.